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Systems Engineering of CHO Cell Line for Enhanced Process Robustness

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Recent advances in genome engineering have opened great opportunities for engineering Chinese hamster ovary cells. An ideal cell line is no longer just one with high productivity, but also with high stability in both the productivity and product quality, and in both extensive passaging and long term continuous culture. Furthermore, an ideal cell line should be provided with process controllability, allowing the use of environmental control variables to steer its metabolism to a reaction pathway that favors the synthesis of product with the desired quality attributes. Importantly, these superior traits must be genetically and epigenetically stable and be passed on to new production lines in cell line development. We have taken a systems approach that integrates genomic information and metabolic model predictions to devise a strategy and to develop tools for attaining those goals. In tool development we reassembled the Chinese hamster genome and combined different versions of the genome to identify consensus segments as high confidence regions and annotated the genome. An expression microarray and a comparative genomic hybridization array for gene coding regions were designed to facilitate cell engineering studies. Using solution phase capture and nested PCR we also established methods of rapidly identifying the integration sites of transgenes on the genome. An induced pluripotent stem cell (iPSC) line was derived from Chinese hamster embryonic fibroblasts for use as control in genomic and epigenomic tool development. Furthermore, we extended our kinetic model for cell metabolism to link with the glycosylation model and now embark on devising a reduced model suitable for systems optimization.

The study involves surveying an established producing line and creating cell lines with a single copy transgene of GFP reporter or of IgG-GFP. The design of the single copy line entails a swappable recombination site for exchange of transgene so that cell lines which are otherwise "identical" but with different transgenes can be systematically compared. Through meta-analysis of archived transcriptome data we identified genes with different dynamics of expression patterns that can be useful for the dynamic control of cell behavior. CRISPR/Cas9 was employed to knock in a GFP between the first exon of the *TXNIP* gene and its endogenous promoter. Interestingly, the transcript levels of GFP in all investigated clones fell in a small range, but the dynamic profile was variable among them. In single copy clones of GFP reporter and IgG transgene, the transcript levels varied widely reflecting the probabilistic nature of their integration in the genome. The IgG titer and their transcript level of the clones also varied over a wide range. Overall, the result does not reveal a correlation between the transgene transcript level and the expression of the gene at the locus. Importantly, a number of clones showed a very high IgG transcript level, consistent with our previous report of a high transgene level prior to transgene amplification. The genomic context that may contribute to the variability, e.g. genomic consistency among the clones in terms of chromosome number, karyotype, and CGH, is being investigated.

The implications of our findings to date and our work on implementing metabolic model prediction in this designing CHO cell line will be discussed. Although this work represents only an early step toward system engineering to cell line development, we believe such approaches will open new avenues to engineer cell lines and influence process development of biologics manufacturing in the coming years.