GENERATION OF A CHINESE HAMSTER OVARY CELL GENOME-WIDE DELETION LIBRARY

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Nowadays, around 70% of all industrially produced biopharmaceuticals are generated from Chinese Hamster Ovary (CHO) cells showing the high interest for further characterization and optimization of this cell line and its derivates. Despite their importance, the connection between the CHO cell genome sequence and function has not been explored in detail so far. Forward genetic screens are the state-of-the-art approach to investigate the link between genotype and phenotype using the CRISPR system as an efficient tool for this purpose. These screens are usually focusing on the ~ 28,000 protein coding genes, which cover only ~ 3 % of the genome. Our approach aims to correlate larger functional regions of the genome, including coding and non-coding sequences, with process relevant cell behavior, such as growth and productivity. To this end, we designed a deletion library approach that targets larger genomic regions of 100 – 150 kb using paired CRISPR gRNAs. So far, we demonstrated successful and efficient deletions up to 150 kb, resulting in proper loss-of-function mutations. These modifications were analyzed on genome and phenotype level, demonstrating that deletion efficiencies are size independent. Furthermore, to enable the presence of active gRNA pairs in each individual cell, we implemented bicistronic transcription of gRNAs separated by a tRNA sequence that unequivocally links each pair. Additionally, we determined CRISPR Cpf1 – an alternative CRISPR enzyme – activity in CHO with no cross-interaction to the CRISPR/Cas9 system, providing the possibility to use the two systems in parallel, one for targeted insertion of the gRNA pair into the genome for later identification of the deleted region, the other for deletion of the corresponding genomic region itself. Currently we are working on the generation of a first smallscale deletion library targeting lncRNAs in CHO for the implementation of the strategy before going genomewide. This will then open the opportunity both of generating large scale gene knockout libraries and of characterizing non-coding genomic regions, gene clusters or regulatory elements.