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IMPLEMENTATION AND EVALUATION OF A HIGH-THROUGHPUT SIRNA SCREENING SYSTEM FOR SUSPENSION CHO CELLS

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Chinese Hamster Ovary (CHO) cells are the most frequently used mammalian cell factory for the production of human-like recombinant proteins. Due to existing limitations in growth and protein production, genetic optimization of CHO cell lines may significantly enhance bioprocess productivities. Knockdown of genes by siRNAs is a standard method to identify genes involved in a desirable phenotype, either because their knockdown improves or degenerates the property. As at least 13000 different transcripts are present in a cell at any time, it is of interest to develop a method that is able to efficiently test the effect of gene knockdown at an appropriate throughput and scale.

Here we describe the implementation of a high-throughput and small scale siRNA screening assay for suspension CHO cells that produce a secreted fluorescent protein. First, growth of CHO cells in 384 well plates was optimized. Second, a suitable method to deliver siRNAs into CHO cells was implemented and optimized. The optimization procedure was conducted by varying initial cell number, lipofection reagent concentration, media composition and incubation time with the help of several control siRNAs. Laser cytometry was used to detect the number of cells, the amount of fluorescent protein per cell and the total fluorescence per well. In addition, cell viability was determined afterwards by the CellTiter Glo® Luminescent Cell Viability Assay. The screening system was evaluated by a pilot screen, consisting of a set of kinome-targeting siRNAs (n=2112). For assessment of reproducibility, this entire screen was conducted twice.

While the viability assay shows bad reproducibility, questioning its suitability, the cell number, amount of fluorescent protein per cell, and the total fluorescence per well show a good correlation between the two screens. Target genes, capable of enhancing the phenotype of CHO cells towards a higher growth and/or productivity upon their siRNA-induced knockdown were identified. This indicates the suitability of this high-throughput siRNA screening system to identify genes that are involved in the enhancement of growth and/or productivity in CHO cells.