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Developing a CRISPR/Cas9 screening platform for Chinese Hamster Ovary cells

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K. J. L. C. Karottki[I], S. Li[I], L. E. Pedersen[I], H. Hefzi[II], P. Spahn[II], A. Thomas[II], J. S. Lee[I], G. M. Lee[III], N. E. Lewis[II], H. F. Kildegaard[I]

[I]DTU, lyngby, Denmark, [II]UCSD, San Diego, United States of America, [III]KAIST, Daejeon, South Korea

Chinese hamster ovary (CHO) cells are the most common mammalian cell line used for producing bio-therapeutic proteins and serve as the expression system of choice for most best-selling biologics. Although these cells are widely used, the genetic bases underlying desirable phenotypes remain difficult to elucidate. CRISPR/Cas9-mediated genome engineering has proven effective in CHO cells in smaller formats, yet rational target identification on a high throughput level remains a bottleneck. CRISPR screening methods have been used in human cancer cell lines to identify novel therapeutic targets but their efficacy in CHO cells for identifying targets for improved cell behavior has not yet been demonstrated. Here we develop a CRISPR/Cas9 knockout screening platform in CHO cells, enabling high throughput target discovery under diverse selection pressures.

We have designed a library comprising ~16,000 gRNAs against ~2500 metabolic targets using the CHO-K1 genome and genome scale model of CHO cell metabolism. The library was used to generate a pool of cells each expressing a single gRNA. As a proof of concept, we have independently subjected the pool of cells to strong and weak selection pressures to identify genes that influence cell growth and product quality. We will discuss the unique challenges encountered in translating CRISPR/Cas9 screens to CHO cells - including technical optimizations during generation and validation of the library that are necessary to ensure the accurate identification of targets. Furthermore, we will present preliminary results from our initial selection experiments.