GENOME-WIDE CRISPR-CAS9 SCREEN IDENTIFIES HYPEROSMOTIC STRESS RESPONSIVE GENES IN CHINESE HAMSTER OVARY CELLS

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Chinese hamster ovary (CHO) cells are the preferred mammalian host cells for therapeutic protein production and have been engineered to contain desired attributes for increased therapeutic protein production. To identify novel engineering targets, laborious and time-consuming empirical approaches have been attempted. In this study, we established a genome-wide CRISPR-Cas9 screening platform for CHO cells with 111,651 gRNAs targeting 21,585 genes using a virus-free, recombinase-mediated cassette exchange-based gRNA integration method. Using this platform, we conducted positive selection screen in hyperosmotic stress condition (463 ± 4 mOsm/kg), which naturally occurs during fed-batch cultures. We identified hyperosmotic stress responsive gene clusters in biological processes by performing functional enrichment analysis using enriched genes in the screen. In addition, we validated the 32 top-scoring genes whose gRNAs showed the most marked enrichment in the screen. The perturbation of genes involved in tRNA modification conferred resistance to hyperosmotic stress. Knockout of putative novel target genes in monoclonal antibody (mAb)-producing recombinant CHO (rCHO) cells and bispecific antibody (bsAb)-producing rCHO cells also enhanced resistance to hyperosmotic stress and increased mAb and bsAb production. The collective findings demonstrate the value of the screen as a platform to infer biological insights associated with hyperosmotic stress and to discover novel targets for rational cell engineering on a genome-wide scale.

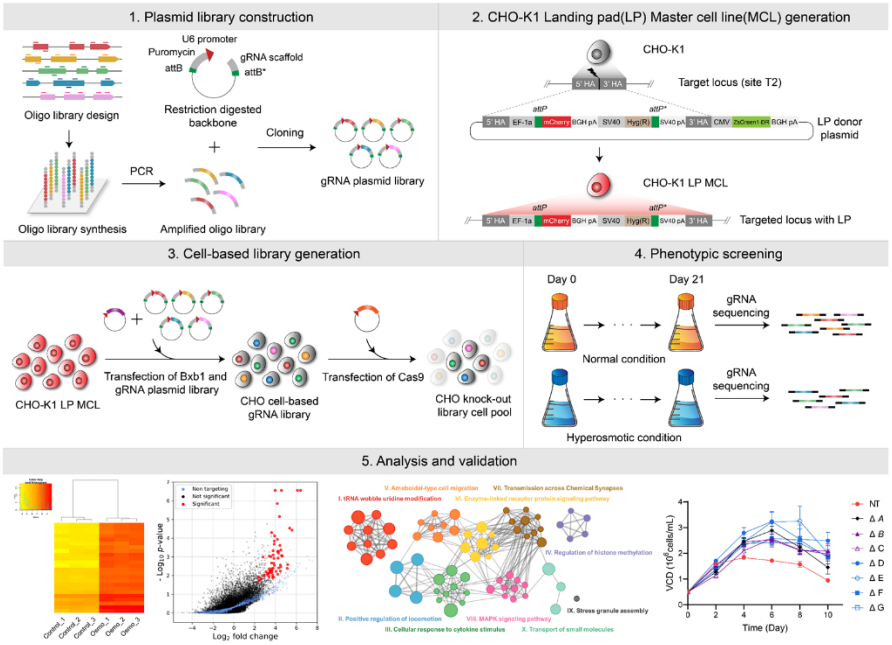

Figure 1 – Overview of genome-wide CRISPR-Cas9 screening in Chinese hamster ovary (CHO) cells