One-step generation of triple knockout CHO cell lines using CRISPR/Cas9 and fluorescent enrichment.

The CRISPR/Cas9 genome editing technology has previously been shown to be a highly efficient tool for generating gene disruptions in CHO cells. In this study we further demonstrate the applicability and efficiency of CRISPR/Cas9 genome editing by disrupting FUT8, BAK and BAX simultaneously in a multiplexing setup in CHO cells. To isolate Cas9-expressing cells from transfected cell pools, GFP was linked to the Cas9 nuclease via a 2A peptide. With this method, the average indel frequencies generated at the three genomic loci were increased from 11% before enrichment to 68% after enrichment. Despite the high number of genome editing events in the enriched cell pools, no significant off-target effects were observed from off-target prediction followed by deep sequencing. Single cell sorting of enriched multiplexed cells and deep sequencing of 97 clones revealed the presence of four single, 23 double and 34 triple gene-disrupted cell lines. Further characterization of selected potential triple knockout clones confirmed the removal of Bak and Bax protein and disrupted fucosylation activity as expected. The knockout cell lines showed improved resistance to apoptosis compared to wild-type CHO-S cells. Taken together, multiplexing with CRISPR/Cas9 can accelerate genome engineering efforts in CHO cells even further.

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