Genome editing has become an increasingly important aspect of Chinese Hamster Ovary (CHO) cell line engineering for improving production of recombinant protein therapeutics. Currently, the focus is directed toward expanding the product diversity, controlling and improving product quality and yields. In this chapter, we present our protocol on how to use the genome editing tool Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) to knockout engineering target genes in CHO cells. As an example, we refer to the glutamine synthetase (GS)-encoding gene as the knockout target gene, a knockout that increases the selection efficiency of the GS-mediated gene amplification system.