Chinese hamster ovary (CHO) cells are the most widely used production host for therapeutic proteins. With the recent emergence of CHO genome sequences, CHO cell line engineering has taken on a new aspect through targeted genome editing. The bacterial clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system enables rapid, easy and efficient engineering of mammalian genomes. It has a wide range of applications from modification of individual genes to genome-wide screening or regulation of genes. Facile genome editing using CRISPR/Cas9 empowers researchers in the CHO community to elucidate the mechanistic basis behind high level production of proteins and product quality attributes of interest. In this review, we describe the basis of CRISPR/Cas9-mediated genome editing and its application for development of next generation CHO cell factories while highlighting both future perspectives and challenges. As one of the main drivers for the CHO systems biology era, genome engineering with CRISPR/Cas9 will pave the way for rational design of CHO cell factories.