Chinese hamster ovary (CHO) cells are the most widely used mammalian hosts for production of therapeutic proteins. However, development of recombinant CHO cell lines has been hampered by unstable and variable transgene expression caused by random integration. Here we demonstrate efficient targeted gene integration into site-specific loci in CHO cells using CRISPR/Cas9 genome editing system and compatible donor plasmid harboring a gene of interest (GOI) and short homology arms. This strategy has enabled precise insertion of a 3.7-kb gene expression cassette at defined loci in CHO cells following a simple drug-selection, resulting in homogeneous transgene expression. Taken together, the results displayed here can help pave the way for the targeting of GOI to specific loci in CHO cells, increasing the likelihood of generating isogenic cell lines with consistent protein production.