MicroRNAs with their unique ability to target hundreds of genes have been highlighted as powerful tools to improve bioprocess behavior of cells. The common approaches to stably deplete miRNAs are the use of sponge decoy transcripts or shRNA inhibitors, which requires the introduction and expression of extra genetic material. As an alternative, we implemented the CRISPR/Cas9 system in our laboratory to generate Chinese hamster ovary (CHO) cells which lack the expression of a specific miRNA for the purpose of functional studies. To implement the system, miR-27a/b was chosen as it has been shown to be upregulated during hypothermic conditions and therefore may be involved in controlling CHO cell growth and recombinant protein productivity. In this chapter, we present a protocol for targeting miRNAs in CHO cells using CRISPR/Cas9 and the analysis of the resulting phenotype, using miR-27 as an example. We showed that it is possible to target miRNAs in CHO cells and achieved ≥80% targeting efficiency. Indel analysis and TOPO-TA cloning combined with Sanger sequencing showed a range of different indels. Furthermore, it was possible to identify clones with no detectable expression of mature miR-27b. Depletion of miR-27b led to improved viability in late stages of batch and fed-batch cultures making it a potentially interesting target to improve bioprocess performance of CHO cells.