

C6-17

Step 3: Host cell characterization and optimization

To avoid mycoplasma contamination in cell cultures, testing the new cell lines which are obtained from an outside source using EZ-PCR Mycoplasma Test kit (Biological Industries) according to manufacturer's protocol.

Step 4-1: Transfection

Check to make sure the cells should be 60-80% confluent prior to transfection. The transfection efficiency varies according to cell type and the transfection reagent used. The optimal conditions must be determined empirically. It is highly recommended to test a range of CRISPR/Cas9 plasmid concentration prior to transfection.