Transfection of RNP complex using *Sp*Cas9 protein and sgRNA

For RNP electroporation into cells

1. Prepare an RNP complex of Cas9 protein and sgRNA.
    a. For Nucleofection using 20 ul reaction volume with 32 strip kit, mix 5 ug Cas9 protein (range: 1~10 ug) and 1.5 ug of sgRNA (2.5~10 ug) for $2x10^5$ cells.
    b. For Neon using 10 ul tip for 24 wells, mix 1 ug Cas9 protein (range: 0.5~5 ug) and 0.3 ug of sgRNA (0.25~2.5 ug) for $5x10^5$ cells.
    c. For KI with donor plasmid, include 1~2 ug in transfection mixture.
2. Incubate on ice for 20 min.
3. Add to the cells.
4. Continue with manufacturer's recommendation for electroporation.

For lipid-based transfection

1. In a microcentrifuge tube, combine 1 ug of Cas9 protein (range: 0.5–2 ug) and 0.5 ug of sgRNA (range: 0.25–1 ug) for cells in a 24-well plate. Incubate for 5 minutes.
2. Add 2 ul of Lipofectamine or another lipid-based transfection reagent to 25 ul of OPTI-MEM.
3. Mix RNP complex and OPTI-MEM containing Lipofectamine. Incubate for 5 minutes.
4. Gently add the mixture to the cells.

* The optimal condition for transfection needs to be empirically determined for each cell line. In general, the more Cas9 protein and sgRNA are used, the better the efficiency is. Toxicity and off-target effect should be minimal with Cas9 protein.