# Injection of RNP complex into one cell embryo

1. Mix sgRNA and Cas9 protein at 1:2 ratio by the amount (or 2.5:1 by molar ratio) at highest concentration possible without dilution. Incubate on ice for 10 min.
2. Mix into injection buffer (for example, 10 mM Tris, pH 7.4). Recommended final concentration is shown below. Pronuclear injection is recommended but cytoplasmic injection also works.
    a. For mouse and rat, use 25 ng/ul of sgRNA (range: 10~100 ng/ul) and 50 ng/ul of Cas9 protein (range: 20~200 ng/ul).

       Recommended donor DNA for the knock-in purpose is 50~200 ng/ul.
    b. For zebrafish, use 250 ng/ul of sgRNA (range: 100~400 ng/ul) and 500 ng/ul of Cas9 protein (range: 200~800 ng/ul).
    c. For invertebrate, use 300 ng/ul sgRNA (range: 150~800 ng/ul) and 600 ng/ul Cas9 protein (range: 300~1,600 ng/ul).
3. Cas9 protein is well tolerated in mouse and zebrafish at a high concentration up to 350 ng/ul or 800 ng/ul respectively. In general, a higher amount of sgRNA and Cas9 protein will increase gene editing event however non-specific cutting can also increase.