Protocols for CRISPR sgRNA

For genome engineering in cultured cells and animal embro injection

1. Reconstitution of lyophilized sgRNA

a) Dissolve lyophilized guide RNA in nuclease-free water

b) Recommended stock solution: 2-5 ug/ul

c) Store sgRNA stock solutions at - 80°C

2. Validation of sgRNA activity

a) Set up reaction mix:

Components	Amount
Cas9 protein	500ng
sgRNA	250ng
Targeting substrate	80~200ng
10 x NEB Buffer 3.1	1µl
10 x BSA	1µl
DW	to 10µl

b) Incubate the reaction mixture at 37℃ for 1 hr
c) Add 1 ul of RNase and incubate for 15 min at 37℃
d) Add 1 ul of STOP solution (30% glycerol, 1.2% SDS, 250 mM EDTA (pH 8.0)] to the reaction mixture and incubate for 15 min at 37℃
e) Analyze on 2% agarose gel

3. Gene Knockout Cell Establishment

The amount of the reagents given in the protocol below are for one well of a 24-well plate.
For other reaction formats, scale the amounts of reagents up or down accordingly.

a) Add Cas9 RNP complex (0.5 µg of Cas9 Nuclease and 250 ng of CRISPR sgRNA) to 50 µl Opti-MEM I Reduced Serum Medium

b) In a separate tube, dilute the transfection reagent by adding 4 µl of Lipofectamine 2000 transfection reagent to 50 µl of Opti-MEM I Reduced Serum Medium
c) Mix gently and incubate for 5 min at RT

d) Add the diluted transfection reagent to the tube containing Cas9 protein/sgRNA RNP complexes and mix gently

e) Incubate at room temperature for 20 minutes to allow the formation of Cas9/sgRNA complex

f) Add the Cas9/sgRNA complex to cells to be transfected (e.g. $1x10^5$ NIH3T3)

g) Swirl the plates gently to allow the mixing of the transfection mixture with the medium

h) Incubate the plate at 37°C in a humidified CO2 incubator in a cell culture incubator for 2~3 days

i) Assay samples to determine the genome editing efficiency by T7E1 assay or targeted deep sequencing

4) General guidelines for the establishment of a KO animal by direct injection of CRISPR RNP complex into a one-cell mouse embryo

a) Make a mixture of Cas9 protein and sgRNA:

Cas9 at 20 ng/ul and sgRNA 10 ng/ul (1 ul each) are the recommended concentrations for mouse embryos.

b) Incubate for 15 min at 37℃

c) Inject diluted solution into one-cell mouse embryos