In vivo genome editing with Cas9 nuclease and sgRNA

Overview:

This protocol describes how to edit host cell genome *in vivo* using OriGene Cas9 protein (TP790148) and a synthetic guide RNA (sgRNA).

Required Materials:

- Cas9 Nuclease, S. pyogenes (Cat. No. TP790148, 20 µM)
- sgRNA containing the targeting sequence (GATGCAGCCGTTCTGGAAGC) turboRFP (tRFP) gene
- Genetically modified HEK293 cells with the genome insertion of tRFP expression cassette
- Nuclease-free 1X TE buffer (Tris-EDTA pH7.4)
- Transfection reagent: Lipofectamine® RNAiMAX Transfection Reagent (Thermo Fisher Cat No. 13778100)
- Opti-MEM Media® , Reduced Serum with Glutamax™ (Thermo Fisher Cat No. 51985091)
- Sterile 96-well tissue culture plates

Before Starting

- We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination.
- Dissolve sgRNA oligos in nuclease-free 1X TE buffer (Tris-EDTA pH7.4) to create a 10µM (10 pmol/ul) working stock.

Procedure

1. Form Cas9:sgRNA RNP complexes: For each transfection, mix 2µl (20 pmol) of sgRNA with 1µl (20 pmol) of Cas9 nuclease (Cat. No. TP790148, 20 µM). Add Opti-MEM Media to a final volume of 12.5 µl.
   Note that you may need to experimentally determine the optimum Cas9:sgRNA ratio for your cell type.
2. Incubate at room temperature for 15 minutes to assemble the RNP complexes.
3. To form each Transfection Complex, incubate the followings at room temperature for 30 minutes (perform step 4 during incubation).

Component	Volume (25 µl final)
RNP Complex (from step 2 above)	12.5 µl
Lipofectamine RNAiMAX Transfection Reagent	1.2 µl
Opti-MEM Media	11.3 µl
Total reaction volume	25 µl

4. During the 30-minute incubation, dilute cultured cells (tRFP positive genetically modified HEK293 cells) to approximately 4 X 10^5 cells/ml using complete media with no antibiotics.

5. Following incubation, add the 25 µl Transfection complex (from step 3) to each well of a sterile 96-well tissue culture plate

6. Add 125 µl diluted cells (from step 4) to each well of the 96-well tissue culture plate. Each well should contain approximately 5 X 10^4 cells and 10 nM of RNP complex.

7. Incubate the plate in the tissue culture incubator at 37°C (5% CO_2) for 48~72 hours

8. Determine the editing efficiency by observing the tRFP signal under microscope.

Result

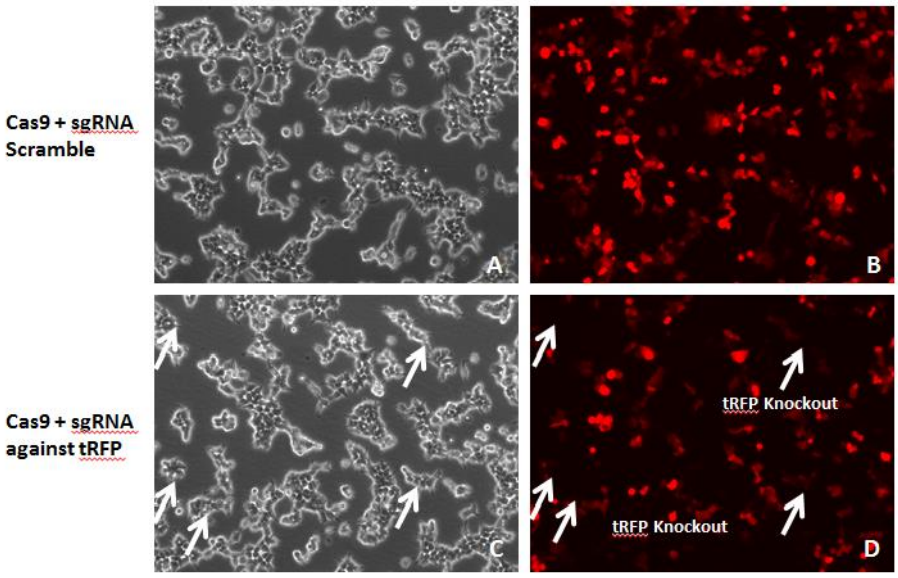

Figure 2: In-vivo genome editing of tRFP positive cells with purified Cas9 protein (Cat. No. TP790148) and specific sgRNA. Purified Cas9 protein can knockout the expression of tRFP protein in the tRFP postive cells when co-transefected with specific sgRNA.