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Generation of CRISPR/Cas9 edited mammalian cells with small epitope tags

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We use this protocol and it's working

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Abstract

This protocol explains how to endogenously edit immortalized mammalian cell culture lines to engineer an in-frame small epitope tag into a gene of interest.

Materials

- Alt-R™ CRISPR-Cas9 crRNA (synthesized by IDT)
- Alt-R™ CRISPR-Cas9 tracrRNA (IDT, Cat# 1075934)
- Alt-R™ S.p. HiFi Cas9 Nuclease V3 (IDT, Cat# 1081060)
- Nuclease-free duplex buffer (IDT, Cat# 11-01-03-01, included with Alt-R CRISPR-Cas9 tracrRNA)
- Single stranded repair oligo (IDT)
- PCR tube
- Thermal cycler
- 1xPBS
- Trypsin-EDTA (0.25%), phenol red
- 15 mL conical tube
- 1.5mL microcentrifuge tubes
- Microcentrifuge
- Lonza 4D Nucleofector System Core Unit with X Unit
- Lonza nucleofection solution
- Lonza nucleofector cuvette
- DMEM complete medium (10%FBS, 1% Pen/Strep, 0.2% Plasmocin)
- 6-well cell culture plate
- HDR enhancer (IDT, Cat# 10007910)

Assemble the gRNA

15m

1 Resuspend Alt-R CRISPR-Cas9 crRNA and Alt-R CRISPR-Cas9 tracrRNA in nuclease-free duplex buffer at 200 µM

2 Mix 5 µl of 200 µM crRNA and 5 µl of 200 µM tracrRNA together in a PCR tube

2.1 Heat to 95°C for 5 minutes in a thermal cycler 5m

2.2 Let cool to RT 10m

Assemble the RNP

30m

3 In a microcentrifuge tube, mix 1.7 µl (104 pmol) of Alt-R S.p. Cas9 nuclease together with 1.2 µl (120 pmol) Alt-R CRISPR-Cas9 crRNA/tracrRNA duplex and 2.1 µl of 1xPBS solution.

3.1 Incubate for 30 minutes at RT 30m

Prepare repair oligo

4 Resuspend repair oligo in 1xPBS at a concentration of 100 µM (pmol/µl).

Prepare cell & electroporation

10m

5 Lift A549 cells from dish using trypsin digest and collect in a 15 mL conical 5m

6 Count cells and pellet ~800,000 cells in a microcentrifuge tube at 300g for 3 minutes. 5m

7 Resuspend cell pellet in 100 µL desired Lonza nucleofection solution

8 Add 2 µL of repair oligo (100 µM) to 5 µL of pre-assembled RNP complex from Step 3

9 Add RNP complex and repair oligo to resuspended cells in nucleofector cuvette

10 Electroporate cells according to Lonza nucleofection kit specifications

Post electroporation

11 Remove cells from nuclofector cuvette and directly add to 2mL warmed complete culture media in one well of a 6-well plate

12 Add 3.4 µL of HDR enhancer to the 2 mL culture (optional)