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CRISPR RNP Electroporation Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.8yfhxtn

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Culture Cells

1 **Subculture cells for a minimum of 2-3 days before electroporation, and visually inspect the cells with a microscope to ensure healthy appearance.**

2 Resuspend Oligos in IDTE Buffer at 100 uM
For a 2nmol IDT order, dilute oligos with 20 uL of Tris-HCL (Dharmacon)

Form the RNP Complex

3 For each well-undergoing electroporation, dilute the guide RNA and Cas9 enzyme in PBS gently swirling the pipet tip while pipetting:
1.3 uL of STERILE PBS
5.1 uL of **GenCrispr NLS-Cas9-EGFP Nuclease**
3.6 of diluted sgRNA oligos

Total: 10 uL of total volume

4 Incubate for 15 minutes at Room Temperature
00:15:00

Prepare Nucleofector System

5 Turn on the nucleofector and load the X-core and the program of the desired cell type (or closest cell type)

Resuspend the Alt-R Cas9 Electroporation Enhancer

6 Resuspend the Alt-R Cas9 Electroporation Enhancer to 100 uM in IDTE.

Prepare the Sorting Plate

7 Prepare a 96 well culture plate to receive cells following Nucleofection
(Ideally U Bottom plate and at least 175 uL of culture media) and prewarm to 37 degrees C.

Prepare the Quenching Plate

8 Prepare a 24 well plate with at least 1 mL of media and prewarm to 37 degrees C

Collect Cells

9 Collect 1M cells per electroporation and MAKE SURE TO WASH THE CELLS WITH PBS.
Centrifuge the cells at 30g for 10 minutes at RT

10 Remove as much supernatant as possible without perturbing the pellet.

Electroporation

11 Resuspend the cell pellet in 96 uL of the Ingenio® Electroporation media
Add 4 ul of the diluted electroporation enhancer
Add 10 uL of the RNP gRNA mix to the well

Zap Step

12 Load solution into the Nucleofector module and make sure there are no air bubbles.
Zap and keep in the zap mixture for 10 seconds before removing

Harvest

13 Remove the cells from the cuvette and add the cells to the quenching plate for a minimum of 4-5 hours

FACS Sorting

14 Take samples to be sorted.