Cas9 RNP nucleofection for cell lines using Lonza 4D Nucleofector Version 2

Mark Dewitt & Julia Wong

Abstract

Citation: Mark Dewitt & Julia Wong Cas9 RNP nucleofection for cell lines using Lonza 4D Nucleofector. **protocols.io** dx.doi.org/10.17504/protocols.io.hdjb24n
Published: 17 Mar 2017

Protocol

Step 1.
Bring 100 pmol of Cas9 to a final volume of 5 µL using Cas9 buffer (20 mM HEPES-KOH pH 7.5, 150 mM KCl, 10% glycerol, 1 mM TCEP). For 40 µM stock: 2.5 µL.

Prepare RNP mix

Step 2.

Prepare RNP mix

Step 3.
Add Cas9 to sgRNA slowly while swirling pipette tip, should take 30s to 1 minute.

Prepare RNP mix

Step 4.
Allow RNP to form for 10-20 minutes.

DURATION
00:20:00

Prepare Cells

Step 5.
Count cells. (Trypsinize as needed.)

Prepare Cells

Step 6.
For each nucleofection, pipette 200k cells into a 15 mL conical.

Prepare Cells

Step 7.
Spin 100 x g for 10 minutes to pellet cells softly. While the cells are spinning, prepare plate and cuvette.

Prepare Cells

Step 8.
Prepare a 12-well-plate with 1mL media per well, and pre-warm in the incubator.

Nucleofection

Step 9.
Prepare and label wells on 20uL nucleofection strips. Configure Lonza 4d using recommended cell-type program.

Nucleofection

Step 10.

Pipette off media from cells, gently but completely, using a P200. The pellet is very soft so be careful.

Nucleofection

Step 11.

Resuspend cells in 20 µL of nucleofector solution (usually SF media) using a P200.

Nucleofection

Step 12.

Add the entire 10 µL RNP mix to the 20 µL resuspension and mix.

Nucleofection

Step 13.

Add 1uL of 100uM donor DNA (100 pmoles) and mix well.

Nucleofection

Step 14.

Add nucleofection mixes to the multiwell cuvette, and cap. Pay attention to the orientation of the cap and cuvette in the nucleofector, which is noted in the manufacturer's instructions.

Nucleofection

Step 15.

Insert cuvette into nucleofector and zap.

Nucleofection

Step 16.

Allow cells to sit in nucleofection strips for 10 minutes post-nucleofection. This is supposed to increase efficiency.

DURATION

00:10:00

Step 17.

Nucleofection

Step 18.

Nucleofection

Step 19.

Allow cells 24 hours to settle and recover before attempted downstream analysis. Consider including un-zapped controls to test viability.

DURATION

24:00:00