Cas9/sgRNA ribonucleoprotein nucleofection using Lonza 4D nucleofector

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Abstract

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Protocol

Prepare cells (part 1)

Step 1.

Trypsinize cells and spin down at 100 x g for 5 minutes.

Prepare cells (part 1)

Step 2.

Remove trypsin and resuspend cells in an appropriate amount of fresh media.

Prepare cells (part 1)

Step 3.

Count cells. Record the cell concentration (cells/uL). In the meantime, put media containing cells in a 37C water bath.

Prepare ribonucleoproteins (RNPs) mix

Step 4.

Add 2.5 uL of 40mM Cas9 (100 pmol) to 2.5 uL of Cas9 buffer (20 mM HEPES-KOH pH 7.5, 150 mM KCl, 10% glycerol, 1 mM TCEP-can make this ahead of time, aliquot and store at -20C).

Prepare ribonucleoproteins (RNPs) mix

Step 5.

Add 3880 ng of sgRNA (120 pmol, MW32,327g/mol) to Cas9 buffer totaling 5 uL.

Prepare ribonucleoproteins (RNPs) mix

Step 6.

Add Cas9 to sgRNA slowly while swirling pipette tip.

Prepare ribonucleoproteins (RNPs) mix

Step 7.

Incubate at 37C for 10-20 minutes to let RNP form.

Prepare cells (part 2)

Step 8.

For each nucleofection, pipette 200k cells using a P200 or larger into a 1.5 mL tube.

Prepare cells (part 2)

Step 9.

Spin 100 x g for 10 minutes to pellet cells softly.

Prepare cells (part 2)

Step 10.

While the cells are spinning, prepare a 12-well plate containing 1 mL of media per well. Pre-warm at 37C.

Nucleofection

Step 11.

Prepare and label wells on nucleofection cuvettes. To avoid cells staying in nucleofection solution for a long period of time in the subsequent steps, configure Lonza 4D ahead of time using the recommended cell-type program. Use SF cell line program CM-130 for HEK293T cells.

REAGENTS

Lonza Nucleofector 4d AAF-1002X by Lonza

Amaxa SF Cell Line 4D-Nucleofector Kit S (96 RCT) V4SC-2096 by Lonza

Nucleofection

Step 12.

After centrifugation, cell pellets are soft so carefully remove media from cells.

Nucleofection

Step 13.

Resuspend cells in 20 uL of nucleofector solution (SF cell line solution with added supplement for

HEK293T) using a P200.

Nucleofection

Step 14.

Add the entire 10 uL RNP mix to the 20 µL resuspension and mix using a P200.

Nucleofection

Step 15.

If using a repair template, add 1uL of 100uM single-stranded donor DNA (100 pmoles) and mix well.

Nucleofection

Step 16.

Add nucleofection mixes to the multiwell cuvette, and cap.

Nucleofection

Step 17.

Insert cuvette into nucleofector and zap using the configured program.

Nucleofection

Step 18.

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

Nucleofection

Step 19.

Add 80uL of pre-warmed media to each well. Pipette mixture out with a P200 into your pre-warmed 12-well plate.

Nucleofection

Step 20.

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