RNP nucleofection for cell lines using Lonza 4D Nucleofector

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Abstract

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Protocol

Prepare RNP mix

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.

NOTES
Jacob Corn 12 Aug 2015
Preparing RNP mix notes: Cas9-NLS is stored in -80, sgRNAs are prepped by runoff transcription, Cas9 buffer is kept in the TC hood and must be kept sterile

Prepare RNP mix

Step 2.
Bring 120 pmol sgRNA to a final volume of 5 µL using Cas9 buffer. This means you will need a minimum sgRNA concentration of 24uM.

Prepare RNP mix

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

NOTES
Jacob Corn 12 Aug 2015
This tip comes from the Doudna lab.

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.

DURATION
00:10:00

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.

NOTES

Jacob Corn 12 Aug 2015
Add carefully to one short side of the well, at an angle. Do not produce any bubbles. Solution need not be completely filling the well as long as there are no bubbles.

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

Nucleofection

Step 17.
Add 80uL of pre-warmed media to each well.

Nucleofection

Step 18.
Pipette mixture out with a P200 into your pre-warmed 12-well plate. This should get the vast majority

of cells, but if you wish, you may wash out the rest with media from the same well, chemistry-style.

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