Cas9/sgRNA ribonucleoprotein nucleofection using Lonza 4D nucleofector Version 6

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Abstract

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Before start

Grow cells to 80-90% confluency. Plate enough cells for 200K cells per nucleofection reaction.

Mg2+ is required for cleavage of DNA by Cas9. Avoid buffer containing high concentration of EDTA as it can chelate Mg2+.

Use SF cell line solution with added supplements as nucleofection solution for HEK293T cells .Don't leave cells in nucleofection solution for a long time as it might be toxic to the cells.

Protocol

Warm up trypsin, media and 1x PBS

Step 1.

Prepare cells (part 1)

Step 2.

Trypsinize cells and spin down at 200 x g for 10 minutes at RT:

-Leave cells in trypsin at 37C for 3-5 minutes.

-Add in warm media to neutralize trypsin.

Note: don't leave cells in trypsin for a long period of time.

Prepare cells (part 1)

Step 3.

Remove media containging trypsin and resuspend cells in an appropriate amount of warm 1x

PBS. This step is critical as trypsin and FBS commonly contain RNAse activity.

Prepare cells (part 1)

Step 4.

Count cells. Record the cell concentration (cells/uL). In the meantime, put solution containing cells in 37C

Form the crRNA : tracrRNA duplex (if needed)

Step 5.

Resuspend RNA oligos (cr and tracr) in IDT duplex buffer to final concentrations of 200 uM.

Form the crRNA : tracrRNA duplex (if needed)

Step 6.

Mix the two oligos in equimolar concentrations to a final duplex concentraition of 100 uM. For example, mixing 1.25 uL of 200 uM crRNA and 1.25 uL of 200 uM tracrRNA yields 2.5 uL of 100 uM guide duplex.

Form the crRNA : tracrRNA duplex (if needed)

Step 7.

Heat at 95C for 5 minutes. Alowing slow cooling to RT by leaving tubes on block before proceeding.

Prepare ribonucleoproteins RNPs mix

Step 8.

Add 100 pmol of Cas9 to 120 pmol of gRNA very very slowly:

-For example, if Cas9 is at 40 uM and gRNA stock at 200 uM, add 2.5 uL of 40 uM Cas9 to 0.6 uL of 200 uM gRNA.

Prepare ribonucleoproteins RNPs mix

Step 9.

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

Prepare cells (part 2)

Step 10.

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

Prepare cells (part 2)

Step 11.

Spin 200 x g for 10 minutes at RT to pellet cells softly.

Prepare cells (part 2)

Step 12.

Carefully remove media off of tubes.

Prepare cells (part 2)

Step 13.

Prepare a 12-well plate containing 1 mL of media per well. Pre-warm at 37C.

Nucleofection

Step 14.

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

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

Lonza Nucleofector 4d AAF-1002X by Lonza

Nucleofection

Step 15.

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

Nucleofection

Step 16.

Resuspend cells in 20 uL of nucleofector solution (SF cell line solution with added supplement for HEK293T) using a P200.

Nucleofection

Step 17.

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

Nucleofection

Step 18.

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

Nucleofection

Step 19.

Add nucleofection mixes to the multiwell cuvette, and cap.

Nucleofection

Step 20.

Insert cuvette into nucleofector and zap using the configured program.

Nucleofection

Step 21.

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

Nucleofection

Step 22.

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

Nucleofection

Step 23.

Allow cells 24 hours - 48 hours to settle and recover before attempted downstream analysis. Consider including un-zapped controls to test viability.